RESEARCH PAPER

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Running head (short title): Transversus abdominis plane block in calves

Ultrasound-guided lateral and subcostal transversus abdominis plane block in calves: a cadaveric study

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Abstract

Objectives To describe and assess the ultrasound-guided transversus abdominis plane (TAP) block feasibility in calf cadavers, to compare two injection volumes and to evaluate possible undesired solution spreads.

Study design Prospective, descriptive, anatomic study.

Animals Fifteen bovine cadavers weighing 47 ± 11 kg, mean ± standard deviation.

Methods Two TAP block approaches were assessed, lateral (n = 24) and subcostal (n = 12). Two volumes, 0.2 or 0.4 mL kg^{-1}, of toluidine blue and contrast medium were injected for each approach using both sides of the animals. Nerve staining was assessed by anatomical dissection; spread of injectate by contrast-enhanced computed tomography. Objective and subjective technique feasibility was evaluated by a specific score (poor, good, excellent).

Results Using the lateral approach, 58, 92 and 25% and 75, 83 and 25% of the thirteenth thoracic, first and second lumbar nerves were stained by 0.2 and 0.4 mL kg^{-1}, respectively. Craniocaudal and dorsoventral solution spread and number of blocks that adequately stained an individual nerve was not significantly different between the volumes. Using the subcostal approach, 67, 83, 67, 67 and 50%, and 83, 100, 83, 83 and 50% of the eighth, ninth, tenth, eleventh, twelfth thoracic nerves were stained by 0.2 and 0.4 mL kg^{-1}, respectively. With both techniques no intra-spinal and one intra-peritoneal spread were observed. Objective and subjective feasibility score was excellent for both approaches in the majority of the cases.

Conclusion and clinical relevance TAP injections were easy to perform with both techniques in calf cadavers. The volume of injectate did not influence spread. Authors conclude that a combination of the two approaches is necessary, but maybe not sufficient, to stain all of the nerves innervating the ventral abdominal wall. Further studies are required to refine the technique and evaluate its efficacy in preventing nociception in calves.

Keywords calves, local anaesthesia, nerve staining, TAP block, ultrasound.

Introduction

Umbilical hernia is the most common congenital disease in calves requiring surgical intervention (Fazili et al. 2013) and general anaesthesia has been recommended (Baird 2008). The anatomical site for median celiotomy in a calf is innervated by the ventral branches of the last thoracic nerves and first (L1) and second (L2) lumbar nerves (Arnold & Kitchell 1957; Budras & Wünsche 2002). During surgery, a skin incision around the umbilicus, subcutaneous tissue dissection and a body wall incision are performed (Baird 2008), inevitably eliciting nociception and acute pain. In humans, pain elicited by the surgical incision is known to significantly contribute to postoperative pain following abdominal surgery (McDonnell et al. 2008). Only few analgesic drugs are allowed by European regulations for use in food-producing animals. Improving local anaesthetic techniques in this species may provide more extensive or consistent perioperative analgesia.

The transversus abdominis plane (TAP) block will interrupt neural transmission in the sensory afferent nerves innervating the abdominal wall. The technique is commonly performed using ultrasound (US)-guidance. In humans, the TAP block decreases postoperative pain, opioid consumption and its related side effects (nausea, vomiting) following abdominal surgeries (Johns et al. 2012; Kitlik et al. 2017; Ma et al. 2017). The anatomy of the TAP block in humans has been studied using cadavers (Barrington et al. 2009; Moeschler et al. 2013), computed tomography (CT; McDonnell et al. 2007; Moeschler et al. 2013) and magnetic resonance (McDonnell et al. 2007). In veterinary medicine, a few studies in dogs (Schroeder et al. 2011; Bruggink et al. 2012; Portela et al. 2014; Drozdzynska et al. 2017), and two case reports, one in a lynx (Schroeder et al. 2010) and one in calves (Zoff et al. 2017a), document its possible usefulness. Conflicting evidence about the effect of the injected volume on the spread of the solution have been reported in both humans (Carney et al. 2011; Moeschler at al. 2013; Forero et al. 2015) and dogs (Bruggink et al. 2012; Zoff et al. 2017b).

The objectives of this study were: 1) to develop an US-guided TAP block technique in calves; 2) to evaluate the spread of two injection volumes of toluidine blue solution by anatomical dissection; and 3) to identify any spread of contrast medium into abdominal cavity, visceral organs and spinal canal using contrast-enhanced CT.

Materials and methods

A total of 15 calf cadavers weighing less than 100 kg were collected between February 2016 and March 2017. All the animals were euthanized for reasons unrelated to the present study. Immediately after death they were frozen and then thawed before the study was performed. Exclusion criteria were presence of abdominal wall abnormalities or history of previous abdominal surgeries. Being a cadaveric study, no committee approval was requested by our institution. All the procedures were performed with the animal in dorsal recumbency.

Sonography of the targeted injection sites was performed by an anaesthesia resident with moderate experience in US-guided locoregional blocks (AM). An US machine (M-Turbo Ultrasound System; Fujifilm SonoSite, WA, USA) equipped with a 6–13 MHz linear array transducer was used. Needles with ultrasound-enhancing features (21 gauge × 110 mm; SonoTAP cannula; Pajunk, Germany) and a solution composed of saline, a staining solution [1% toluidine blue solution containing 1% borax (Toluidinblau O, Carl Roth GmbH, Germany)] and a contrast medium (Accupaque 300; GE Healthcare, Switzerland) in a 2:1:1 ratio were used for bilateral injections. Injection volumes of 0.2 mL kg^{-1} and 0.4 mL kg^{-1} were chosen.

Lateral TAP block

In 12 animals, the TAP block was performed using a lateral approach (24 blocks). Both injection volumes were used on each animal. The starting side (right or left) and the volume were randomized using software available online (www.randomization.com).

Initially, the probe was positioned cranial to the iliac crest and perpendicular to the long axis of the body, as previously described in humans and dogs (El-Dawlatly et al. 2009; Schroeder et al. 2011). From this point, the probe was tilted in the direction of the xiphoid and moved slowly toward the ventral part of the abdomen to obtain better simultaneous visualization of the obliquus externus abdominis muscle, obliquus internus abdominis muscle and transversus abdominis muscle (TAM). Thereafter, the needle was inserted in a craniocaudal direction using an in-plane technique, with a 20° angle. When the interfascial plane located between the obliquus internus abdominis muscle and the TAM was thought to be reached, a test dose of solution (1 mL) was injected to confirm correct positioning. In case of incorrect spread (e.g. intramuscular spread, wrong interfascial plane) the needle was repositioned and another test dose was injected. This procedure was repeated until the correct site was reached. Then, the predefined volume of solution (minus the 1 mL of the test dose injected in the correct place) was injected. Afterward, the technique was repeated on the contralateral side.

Subcostal TAP block

In six animals, the TAP block was performed using a subcostal approach (12 blocks). Both the lateral and subcostal approaches were administered to three of these animals.

The initial probe positioning was just caudal to the xiphoid process, in a transverse orientation, as previously described in dogs (Drozdzynska et al. 2017). At this point the linea alba and the bilateral bodies of the pectoralis major muscle were identified. The probe was moved laterally and simultaneously rotated along the subcostal margin, until a good view of the rectus abdominis muscle and the TAM was obtained. The probe was then slid in a craniocaudal direction until it reached the midpoint between the xiphoid and the last rib. Thereafter, the needle was inserted in a craniocaudal direction using an in-plane technique, with a 20° angle. When the interfascial plane between the rectus abdominis muscle and the TAM was thought to be reached, a test dose of solution (1 mL) was injected to confirm correct positioning. Afterward, the same procedure used for the lateral approach was followed and the technique was repeated on the contralateral side.

Lateral and subcostal TAP block assessment

The quality of US visualization was scored as excellent, good or poor if 3, 2 or 1/none of the following criteria were met: a) muscular layers easily distinguishable (obliquus externus abdominis muscle, obliquus internus abdominis muscle and TAM for the lateral approach, and rectus abdominis muscle and TAM for the subcostal approach); b) needle fully visualized; and c) clear separation of the two muscular layers (obliquus internus abdominis muscle and TAM for the lateral approach, and rectus abdominis muscle and TAM for the subcostal approach) during injection.

Then, the investigator subjectively judged the feasibility of the technique as excellent, good or poor. Finally, the number of attempts to achieve a correct injection was recorded. Possible spread of the solution into the abdominal cavity, visceral organs and spinal canal was assessed by contrast-enhanced CT images analysis. Spread of the solution and staining of the ventral rami from the eighth thoracic nerve (T8) to L2 was assessed by anatomical dissection. The nerve was considered adequately stained if the long axis of the nerve was stained ≥1 cm. CT images analysis and anatomical dissections were always performed by the same investigator (MS and AvonR, respectively).

Statistical analysis

The number of animals included to investigate the lateral TAP block was based on previous anatomical studies in humans and dogs (Tran et al. 2009; Schroeder et al. 2011). After obtaining the first results, a power calculation based on the craniocaudal spread in cm [group 0.2 mL kg^{-1}: mean ± standard deviation (SD) of 7.6 ± 2; group 0.4 mL kg^{-1}: mean of 10.6; using software available online (http://clincalc.com/stats/samplesize.aspx)] was performed. To have a power of 0.8 with an alpha value of 0.05, a total of 14 blocks (7 per group) were needed. Descriptive statistics were used for both the lateral and subcostal TAP block.

Statistical tests were performed using SigmaStat (SigmaStat Version 3.5; Systat Software Inc., CA, USA). Outcome parameters were: craniocaudal spread (cm), dorsoventral spread (cm) and number of blocks (%) that adequately stained an individual nerve. A Kolmogorov-Smirnov's test was performed, revealing not normally distributed data. Differences between the two volumes of injection were evaluated using Wilcoxon signed rank test and Fisher exact test. Statistical significance was set at $p < 0.05$. All p values were corrected for multiple testing by applying Bonferroni-Holm adjustment.

Results

The cadavers were of two breeds [Holstein Friesian (n = 13) and Simmental (n = 2)], 10 males and five females with a mean ± SD weight of 47 ± 11 kg. At the end of the study, a total of 24 lateral and 12 subcostal injections were collected and evaluated. Among these, 12 lateral injections were performed with a volume of 0.2 mL kg^{-1} and 12 with a volume of 0.4 mL kg^{-1}, while 6 subcostal injections were performed with a volume of 0.4 mL kg^{-1} and 6 with 0.2 mL kg^{-1}.

An anatomical dissection displaying the innervation of the ventral abdominal wall of the calf and a three-dimensional CT image showing the diffusion of the contrast medium following bilateral lateral and subcostal injections are available as supporting information online (Figs S1 & S2).

Lateral TAP block

The quality of US visualization, feasibility score, number of attempts, and spread of the solution are reported in Table 1. US visualization was scored as excellent in 22 out of 24 injections and as good in 2 other injections. Feasibility was scored as excellent in 22 out of 24 injections and as good in the 2 other injections. One attempt was sufficient to correctly perform the injection in 21 out of 24 injections, and two and three attempts were required in 2 and 1 injections, respectively.

The number of blocks that adequately stained an individual nerve is reported in Table 2. None of the nerves cranial to T13 were stained by a lateral TAP injection.

One intraperitoneal spread and no intraspinal spread were detected. The dorsal spread of the contrast medium toward the spinal canal was always blocked by the epaxial lumbar musculature (musculus longissimus dorsi). No significant differences were found in the craniocaudal and dorsoventral spread of the solution and in the number of blocks that adequately stained an individual nerve between the two volumes of injectate.

Subcostal TAP block

The quality of US visualization, feasibility score, number of attempts, and spread of the solution are reported in Table 3. US visualization was scored as excellent in 10 out of 12 subcostal injections and as good in the other 2 injections. Feasibility was scored as excellent in 8 out of 12 injections and as good in 4 injections. One attempt was sufficient to correctly perform the injection in 8 out of 12 injections, and two attempts were needed for 4 injections. The number of nerves adequately stained by subcostal injection is reported in Table 2; none of the nerves caudal to T12 were stained. No intraspinal spread and one intraperitoneal spread were detected.

A noteworthy peculiarity of the rectus abdominis muscle at US visualization was the presence of oblique, hyperechoic, regularly distributed lines in its body, that likely represent the tendinous intersections (Fig.1).

Discussion

The present study aimed to develop and evaluate the TAP block technique in calf cadavers. The results indicate that two injections, lateral and subcostal, on each side of the abdomen, are necessary to stain all of the nerves innervating segments of the ventral abdominal wall possibly involved in common median celiotomies.

Both the lateral and subcostal approaches were easy to perform. Indeed, US visualization and feasibility for both approaches were judged as excellent and only one attempt was needed to perform them correctly in the majority of the injections. Moreover, intraperitoneal spread was rarely detected and intraspinal spread of the solution was not observed.

Increasing the volume did not affect the craniocaudal and dorsoventral spread of the solution when the lateral TAP block was performed, or the number of injections that adequately stained an individual nerve. This is in accordance with the studies of Carney et al. (2011) and Forero et al. (2015) in humans and Zoff et al. (2017b) in dogs, in which no differences in solution spread occurred with different injection volumes. Conversely, studies in humans published by Suresh et al. (2015) and Moeschler at al. (2013) and in dogs by Bruggink et al. (2012) identified a direct correlation between injection volume and spread of the solution. In light of the results from the present and the above mentioned studies, further investigations are needed to improve the TAP block technique, aimed at increasing the percentage of nerves stained. If volume is not the limitation of solution spread, combination of the TAP block with a second technique could lead to better nerve staining.

In this study, difficulty was encountered when attempting the subcostal approach during the preliminary pilot trial because the fascia between the rectus abdominis muscle and the TAM was difficult to delineate. This problem arose because the pectoralis major muscle extends quite caudally in calves, it is a large muscle in this species and was initially identified as the rectus abdominis muscle. Another misleading feature was the presence of oblique, hyperechoic, regularly distributed lines in the rectus abdominis muscle body that the authors postulated represented the tendinous intersections. Their regular distribution and oblique direction made them easily recognizable, however, it is important to not consider them as intermuscular fascia when performing this technique.

Sensory innervation of the ventral abdominal wall possibly involved in routine median celiotomy is provided by the last thoracic nerves and the first two lumbar nerves (Arnold & Kitchell 1957; Budras & Wünsche 2002). Based on previous literature (Budras & Wünsche 2002) and on the findings of the anatomical dissections in the present study, the ventral branches of T8 innervate the most cranial abdominal region that could be involved during the surgical procedure. The ventral branches of the T8 to L2 pass over the TAM, providing innervation of the peritoneum, muscular layers, subcutis and skin (Arnold & Kitchell 1957; Budras & Wünsche 2002). Since the incision is performed on the midline, a bilateral nerve block is needed to ensure adequate analgesia. As already suggested by Drozdzynka et al. (2017) and in the light of the present results, a combination of the lateral and subcostal techniques may represent the best option for providing analgesia during median celiotomy, particularly when a long surgical incision is required. For surgeries involving the inguinal area, blockade of the third lumbar nerve (L3) could be necessary. In the present study, L3 was never stained and an additional anaesthetic technique would be required.

The L2 nerve following the lateral approach and the T12 nerve following the subcostal approach were stained only in 25 and 50% of the cases, respectively. Nevertheless, communication between adjacent thoracolumbar nerves has been described in humans (Rozen et al. 2008) and the extent of sensory blockade of the area innervated by these two nerves following the combination of lateral and subcostal TAP block may be still adequate *in vivo*.

In the present study, the soft tissues adhering to the caudal part of the last rib impeded the spread of the solution from the lateral TAP injection site to the intercostal space, as reported in humans (Tran et al. 2009). Thus, staining of the nerves cranial to T13 was not possible solely with a lateral injection, although effectiveness of a higher injection volume, possibly reaching the intercostal nerve exiting the costal arch, cannot be ruled out. However, the potential toxicity of higher volumes of local anaesthetic agents needs to be considered. An initial injection volume of 0.2 mL kg^{-1} block was chosen based on the maximum suggested dosage of procaine 1% (4 mg kg^{-1}) (Rioja Garcia 2015) when the study design initially included only a bilateral lateral TAP block injection. Of relevance, procaine is the only local anaesthetic drug allowed by European and Swiss regulations for use in food producing animals. Doubling the volume was chosen to investigate the influence of volume on the spread of the solution. With the final study design investigating the combination of both lateral and subcostal techniques, a volume of 0.2 mL kg^{-1} for each injection resulted in a total procaine dose of 8 mg kg^{-1}. No known study on the toxic dose of procaine has been performed in calves. In humans, a maximum dose of 500 mg for infiltration anaesthesia has been suggested (Berde & Strichartz 2015) but no side effects have been reported after single intravenous (IV) administration of dosages up to 5000 mg (EMEA/MRL/217/97-FINAL 1998). In dogs, procaine (20 mg kg^{-1}) IV resulted in muscle tremors and incoordination (EMEA/MRL/217/97-FINAL 1998). In horses, procaine (2–10 mg kg^{-1}) administered IV resulted in locomotor, cardiovascular and behavioural reactions similar to the ones described when procaine benzylpenicillin is administered, none of which was fatal (EMEA/MRL/217/97-FINAL 1998).

To overcome a potential overdose, if an increase in the volume of injection is desired, drugs can be diluted. Nevertheless, lower concentrations might be less efficacious and the minimum concentration at which procaine would still provide sensory block is, to date, unknown. Moreover, the role that the volume:concentration ratio has on block success rate, onset time and duration remains controversial in the literature (Taboada Muñiz et al. 2008; Bertini et al. 2009; Cappelleri et al. 2014).

This study has some limitations that need to be considered before implementing the technique in clinical practice: 1) the technique was performed on thawed cadavers at room temperature, therefore, solution spread, ultrasound imaging and extent of sensory blockade may be different *in vivo*; 2) dilution of local anaesthetics might be necessary to obtain a minimal effective injection volume, possibly reducing the analgesic efficacy of the technique; 3) only six cadavers were used to describe the subcostal approach, therefore only descriptive statistical analysis was possible; and 4) only one investigator performed the US-guided block, thus the feasibility of the technique could change depending on operator experience.

In conclusion, the TAP block is a feasible and easy to perform technique in calves. A combination of the lateral and subcostal approaches is recommended to obtain adequate spread of the solution over the sensory nerves supplying the ventral abdominal wall. However, further investigations are needed in order to improve this technique aiming at increasing the percentage of nerves stained. The TAP block could be used in any surgery requiring access to the ventral abdominal wall, and a selective caudal or cranial block could be achieved by using the lateral or subcostal approach, respectively. Injection volume does not seem to influence the spread of the solution when performing the lateral TAP block. Prospective controlled clinical studies in calves are needed to: 1) establish the best volume:concentration ratio of local anaesthetic solution to provide reliable analgesia of the ventral abdominal wall; and 2) to assess the intra- and postoperative analgesic efficacy of the block.

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Authors' contributions

AM: study design, selection of study material, acquisition of data, statistical analysis, preparation of the manuscript; AvonR, MS and LM: acquisition of data, revision and approval of the manuscript; DC: study design, revision and approval of the manuscript; CS: study design, selection of study material, revision and approval of the manuscript.

Conflict of interest statement

Authors declare no conflict of interest.

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Figure S1 Dissection of the left abdominal wall showing the pathway of the eighth thoracic to second lumbar (T8-L2) nerves over the transversus abdominis muscle (TAM) in a calf cadaver. Skin, subcutis, obliquus externus abdominis muscle and obliquus internus abdominis muscle have been removed.

Figure S2 Three-dimensional computed tomography image reconstruction showing the spread of the contrast solution following four ultrasound-guided transverse abdominal plane injections [two lateral (0.2 mL kg^{-1} left and 0.4 mL kg^{-1} right) and two subcostal (0.2 mL kg^{-1} bilaterally)] in a calf cadaver.

Figure 1 Ultrasonographic image obtained while performing the subcostal transversus abdominis plane (TAP) block. The ultrasound probe was positioned parallel to the subcostal margin and slid midway between the xiphoid and the last rib.

RAM, body of the rectus abdominis muscle with its tendinous intersections (arrows); TAM, the transversus abdominis muscle.

Table 1 Lateral transversus abdominis plane (TAP) block. Quality of ultrasound (US) visualization, feasibility of the technique, and number of attempts necessary to perform a correct injection, craniocaudal spread and dorsoventral spread of a solution of toluidine blue and a contrast medium using 2 injection volumes (0.2 and 0.4 mL kg^{-1}) in calf cadavers.

Calf number	Side	US visualization	Feasibility	Number of attempts	Volume (mL kg^{-1})	Craniocaudal spread (cm)	Dorsoventral spread (cm)
1	L	Excellent	Excellent	2	0.2	9	10
	R	Excellent	Excellent	1	0.4	10	12
2	L	Excellent	Excellent	3	0.2	5	4
	R	Excellent	Excellent	1	0.4	20	8
3	L	Excellent	Excellent	1	0.4	8	10
	R	Excellent	Excellent	1	0.2	9	11
4	L	Good*	Excellent	2	0.2	5	10
	R	Good*	Excellent	1	0.4	11	12
5	L	Excellent	Excellent	1	0.2	9	9
	R	Excellent	Excellent	1	0.4	10	15
6	L	Excellent	Excellent	1	0.4	8	10
	R	Excellent	Excellent	1	0.2	12	10
7	L	Excellent	Excellent	1	0.2	7	7
	R	Excellent	Excellent	1	0.4	11	10
8	L	Excellent	Excellent	1	0.4	10	9

	R	Excellent	Excellent	1	0.2	9	11
9	L	Excellent	Excellent	1	0.2	6	9
	R	Excellent	Excellent	1	0.4	8	8
10	L	Excellent	Excellent	1	0.2	9	11
	R	Excellent	Excellent	1	0.4	5	7
11	L	Excellent	Excellent	1	0.2	7	10
	R	Excellent	Excellent	1	0.4	16	10
12	L	Excellent	Good	1	0.2	8	20
	R	Excellent	Good	1	0.4	5	5

L, left side; R, right side. *Separation of muscle layers not clearly visualized during injection.

Table 2 Nerves stained ≥1 cm by lateral or subcostal transversus abdominus plane (TAP) injections using two volumes (0.2 mL kg^{-1} or 0.4 mL kg^{-1}) in calf cadavers.

Spinal nerve	**Injection technique**	**Volume (mL kg^{-1})**	**Number/total (%)**
T8	Lateral	0.2	0/12 (0)
		0.4	0/12 (0)
	Subcostal	0.2	4/6 (67)
		0.4	5/6 (83)
T9	Lateral	0.2	0/12 (0)
		0.4	0/12 (0)
	Subcostal	0.2	5/6 (83)
		0.4	6/6 (100)
T10	Lateral	0.2	0/12 (0)
		0.4	0/12 (0)
	Subcostal	0.2	4/6 (67)
		0.4	5/6 (83)
T11	Lateral	0.2	0/12 (0)
		0.4	0/12 (0)
	Subcostal	0.2	4/6 (67)
		0.4	5/6 (83)

T12	Lateral	0.2	0/12 (0)
		0.4	0/12 (0)
	Subcostal	0.2	3/6 (50)
		0.4	3/6 (50)
T13	Lateral	0.2	7/12 (58)
		0.4	9/12 (75)
	Subcostal	0.2	0/6 (0)
		0.4	0/6 (0)
L1	Lateral	0.2	11/12 (92)
		0.4	10/12 (83)
	Subcostal	0.2	0/6 (0)
		0.4	0/6 (0)
L2	Lateral	0.2	3/12 (25)
		0.4	3/12 (25)
	Subcostal	0.2	0/6 (0)
		0.4	0/6 (0)

Table 3 Subcostal transversus abdominis plane (TAP) block. Quality of ultrasound (US) visualization, feasibility of the technique, and number of attempts necessary to perform a correct injection, craniocaudal spread and dorsoventral spread of a solution of toluidine blue and a contrast medium using 2 injection volumes (0.2 and 0.4 mL kg^{-1}) in calf cadavers.

Calf number	Side	US visualization	Feasibility	Number of attempts	Volume (mL kg^{-1})	Craniocaudal spread (cm)	Dorsoventral spread (cm)
10	L	Excellent	Excellent	1	0.2	13	5
	R	Excellent	Excellent	1	0.2	15	4
11	L	Excellent	Excellent	1	0.2	10	3
	R	Excellent	Excellent	2	0.2	10	5
12	L	Excellent	Good	1	0.2	7	7
	R	Excellent	Good	2	0.2	7	9
13	L	Good*	Good	2	0.4	11	9
	R	Good*	Good	2	0.4	8	8
14	L	Excellent	Excellent	1	0.4	8	5
	R	Excellent	Excellent	1	0.4	15	5
15	L	Excellent	Excellent	1	0.4	12	8
	R	Excellent	Excellent	1	0.4	17	10

L, left side; R, right side. *Muscle layers not easily visualized.